# The selective oestrogen receptor modulators idoxifene and raloxifene have fundamentally different cell-specific oestrogen-response element (ERE)-dependent/independent mechanisms *in vitro*

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**Abstract**

Idoxifene and raloxifene are selective oestrogen receptor modulators (SERMs) that by definition exhibit tissue-specific agonist or antagonist properties via interactions with the oestrogen receptor (ER). Idoxifene acts as an oestrogen agonist in osteoblastic cells via an ER/ERE-mediated mechanism. In contrast, raloxifene is an antagonist via the ERE in osteoblastic cells. Like the pure antagonist ICI 182,780, raloxifene inhibited the potent agonist activity of both 17β-oestradiol and idoxifene through the ERE whereas idoxifene had no effect on the agonist activity of 17β-oestradiol via the ERE. In breast cancer cells, both raloxifene and idoxifene were potent antagonists of ERE-mediated 17β-oestradiol action suggesting an ERE-dependent mechanism of action for both ligands in these cells. Therefore, these SERMs exhibit cell-specific ERE-dependent and -independent mechanisms of action.


*Keywords:* Osteoporosis; Ovariectomy; Idoxifene; Raloxifene; SERM; Oestrogen; Steroid hormone; Bone; Breast

## 1. Introduction

Although clinically useful in preventing bone loss in postmenopausal women, oestrogen therapy is reportedly linked to an increased risk of tissue-specific side-effects including endometrial hyperplasia, which may lead to uterine cancer and proliferative effects in mammary tissue, resulting in an increased risk of breast cancer. The ideal postmenopausal 'oestrogen' would reproduce the beneficial effects of oestrogen on vasomotor symptoms, skeletal tissue and the cardiovascular system, without producing adverse effects on reproductive tissues. This concept has led to the development of SERMs which are defined as compounds that have oestrogen agonism on one or more of the desired target tissues such as bone or liver and has antagonism and/or minimal agonism (i.e. clinically insignificant) in reproductive tissue such as the breast or uterus [1]. We report here fundamental differences in the mechanism of action of two SERMs; idoxifene and raloxifene in bone and breast cells *in vitro*.

## 2. Materials and methods

### 2.1. Cell transfection

The cell lines used in this study were human breast MCF-7 cells, human osteosarcoma (MG-63) and rat osteosarcoma cells (Ros 17/2.8) transfected with either MMTV-ERE-*Luc*, a construct containing five copies of a 33-base pair vitellogenin ERE, upstream of the luciferase reporter gene [2] or the endogenous complement 3 ERE [3] upstream of the luciferase reporter gene. Post-transfection, fresh media was added and cells were incubated for 48 h with or without ligands. Luciferase activity was a measure of ERE-dependent transcriptional activation.

### 2.2. Cell proliferation

MCF-7 cells were treated with ligands (100 nM) for 48 h. Cell counts were performed in triplicate under light microscopy using a haemocytometer.

## 3. Results

Raloxifene and the pure antagonist ICI 182 780 had no demonstrable direct agonist activity on the ERE-luciferase constructs transfected into either human or

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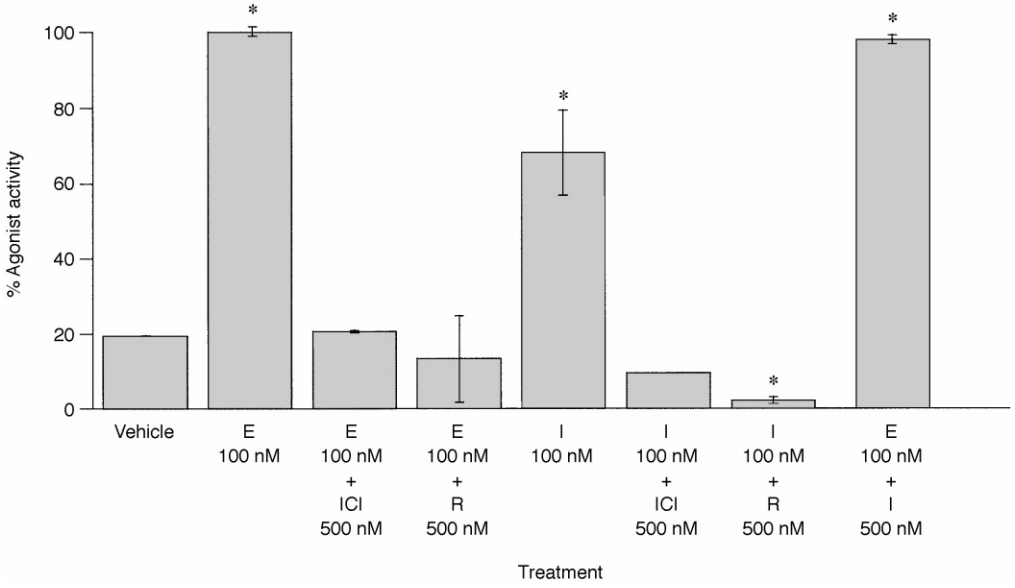


Fig. 1. The effect of oestrogen receptor (ER) ligands on ERE-dependent transcription in rat osteoblast (Ros 17.2.8) cells. I, idoxifene; R, raloxifene; E, oestrogen; ICI, ICI 182,780. Results are expressed as mean standard deviation (S.D.) $n = 3$ ($^*P < 0.05$). Significance was compared with vehicle alone.

rat osteoblasts. In contrast, idoxifene and 17β-oestradiol were both ERE-dependent agonists. In competition experiments, raloxifene and ICI 182 780 (500 nM) inhibited the agonist activity of 100 nM 17β-oestradiol in osteoblasts (Fig. 1). In contrast, idoxifene (500 nM) had no effect on the agonist activity of 17β-oestradiol in osteoblasts. In addition, both raloxifene and ICI 182 780 (500 nM) completely inhibited 100 nM idoxifene-stimulated luciferase activity in osteoblasts, indicating both raloxifene and ICI 182 780 are acting as ERE-dependent antagonists of idoxifene and 17β-oestradiol action.

In contrast to the effects seen in osteoblasts, 17β-oestradiol was the only agonist in human breast cancer cells (MCF-7) transfected with the ERE reporter. Indeed, both idoxifene and raloxifene were both able to completely suppress oestrogen-induced agonism in MCF-7 cells. The lack of agonism of these ER ligands compared with 17β-oestradiol suggests ERE-dependent, cell-specific differences. We also examined human breast cancer cell proliferation which was stimulated by 17β-oestradiol but not by any of the other ligands. None of the ligands had any effect on the proliferation of either rat or human osteoblast-like cells *in vitro*.

Our data indicate that idoxifene functions as an agonist via the ERE in one cell context (bone) and shows no demonstrable agonism in another (breast). These data suggest a mechanism of action comparable to 17β-oestradiol, but distinct from raloxifene in osteoblasts.

## 4. Conclusion

It remains to be determined which of the growing number of potential genomic and non-genomic pathways play critical tissue-specific roles that influence ERE-dependent agonism/antagonism. However, there are clearly mechanistic cell-specific differences exhibited by these ER-ligands, which can result ultimately in the same physiological endpoint.

## References

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